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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall subject of this project is blast-traumatic brain injury (blast-TBI) and the role of the SUR1-regulated NCCa-ATP channel in blast-TBI. The specific objectives of this project include: (1) develop a standardized rat model of blast-TBI to study the direct transcranial effects of blast on the brain, independent of indirect transthoracic effects; (2) determine the role of the SUR1-regulated NCCa-ATP channel in blast-TBI; (3) in normal human volunteers, determine the safety of the SUR1 blocker, glyburide, as it might be used as prophylaxis against blast-TBI. During the 4th year of this project we performed detailed anatomical evaluation of the cells de novo expressing SUR1 protein, and quantitative measurements of the SUR1 and TRPM4 RNA and proteins after blast injury. We compared cellular responses of the brain to direct delivery of the blast wave to the brain via cranial exposure versus indirect delivery of the blast wave to the brain via thoracic exposure. The most important findings include: (i) cranium only (COBIA) blast impacts brain tissues at density boundaries and brain-blood barrier is minimally altered, (ii) after COBIA injury Sur1 and TRPM4 RNA and protein upregulation is detected as early 4 hours after the blast, (iii) thoracic delivery of the blast results in distinct cerebral perivascular inflammation.

15. SUBJECT TERMS
blast-TBI, sulfonylurea receptor

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Table of Contents

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The overall subject of this research project is blast-traumatic brain injury (blast-TBI) and the role of the SUR1-regulated NCCa-ATP channel in secondary injury following blast-TBI. The specific objectives of this research project may be summarized as follows:
(1) develop a standardized rat model of blast-TBI, to permit study of direct transcranial effects of blast on the brain, independent of indirect transthoracic blast effects;
(2) using this rat model, determine the specific role of the SUR1-regulated NCCa-ATP channel in blast-TBI, including testing whether block of SUR1 using glibenclamide would show a beneficial effect in blast-TBI;
(3) in normal human volunteers, determine the safety of oral glibenclamide as it might be used as prophylaxis against blast-TBI.

NOTE: Rather than being final, this report covers research activity only in the time period between 07/01/2011 and 07/02/2012 which reflects requested modification of the grant to extent project for one more year without additional funds.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but must be referenced in the body of the report. If applicable, for each task outlined in the Statement of Work, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included, although changes to the original Statement of Work must be approved by the Army Contracting Officer Representative. This approval must be obtained prior to initiating any change to the original Statement of Work.

During last year of the project we concentrated our efforts on the following tasks:

1) Comparison of cellular responses in the brain regions to direct delivery of the blast wave to the brain via cranial exposure versus indirect delivery of the blast wave to the brain via thoracic exposure.
2) Detailed anatomical evaluation of the cells de novo expressing SUR1 protein Correlation between SUR1 and neuronal injury
3) Quantitative measurements of the SUR1 and TRPM4 RNA and proteins after blast
4) Preparation fro GLP study of the effect of glibenclamide on the blast injury induced cranium-only blast injury apparatus (COBIA).

Summary of animal use:
In the time period between 07/01/2011 and 07/02/2012,
total rats used was 173, including 11 naïve, 21 sham, 10 exclusions, 108 blasted rats with COBIA and 23 rats blasted with TOBIA

Overall, primary blast deaths 38, (including 3 anesthesia death).

From 108 COBIA blast TBI's –
      8 using the 24.5 cm BDC and charge 4 powerload (2 blast deaths)
      20 using the 27 cm BDC charge 4 powerload (9 blast death)
      81 using the 29.5 cm BDC charge 4 powerload (18 blast death)

From 23 TOBIA blast TBI's –
      2 using the 24.5 cm BDC and charge 4 powerload (2 blast deaths)
      21 using the 27 cm BDC charge 4 powerload (4 blast death)

> **Objective 1a:** establish the usable working range for the "intensity-response" relationship between blast intensity and outcome in our blast-TBI model

A detailed description and validation of the Cranium Only Blast Injury Apparatus (COBIA) to deliver blast overpressures generated by detonating .22 caliber cartridges of smokeless powder was published during this year in *J. Neurotrauma*.[1] Our published data demonstrate our central thesis, that exposure of the head alone to severe blast predisposes to significant neurological dysfunction.

Additionally to the data on the COBIA reported in our paper last year we studied more in detail impact of the cranium-only blast on the neuronal injury in the brain stem. We detected upregulation of both of cleaved caspase-3 (Fig. 1), and upregulation of β-APP. Arguably, one of the most intriguing findings was involvement of neurons of the raphe system, which projects 5-HT fibers to cortical and other forebrain regions, and play an important role in neuropsychological function. Cells of the dorsal raphe, identified by their location and by expression of tryptophan hydroxylase, showed upregulation of β-APP (Fig.2,3), Sur1 (Fig. 4,5) and TUNEL labeling (Fig. 6).

We hypothesized that neurons of the raphe system may be selectively vulnerable to blast injury, for the same reason that pial veins appear to be selectively vulnerable – they reside near a CSF-brain boundary. Moreover, raphe neurons located at the rostral end of the 4th ventricle, where the 4th ventricle joins the aqueduct of Sylvius, may be selectively vulnerable because the "bottleneck" at this location accelerates a hydrodynamic pulse traveling upward from the 4th ventricle, transferring kinetic energy to surrounding tissues. Indeed, immunolabeling for Sur1 showed exactly this phenomenon (Fig. 4). Also, the nearby cluster of large raphe neurons showed distinct

upregulation of Sur1 (Fig. 4). The raphe neurons that upregulated Sur1 were identified by their location and by expression of tryptophan hydroxylase (Fig. 5).

These findings of widespread injury to raphe neurons correlates well with data presented below (Fig. 15) on abnormal thigmotaxis, a measure of anxiety, following blast induced by COBIA. Moreover, the involvement of Sur1 in this pathological response correlates with data presented below that abnormal thigmotaxis following blast induced by COBIA was significantly ameliorated by treatment with glibenclamide.

Overall, **direct cranial blast injury (dcBI)**, did not result in widespread or generalized extravasation of IgG, cerebral edema and brain swelling. We speculated that vascular dysfunction would be more likely when kinetic energy from the blast wave is transferred to the brain via the transthoracic/transvascular route. This led us to conduct series of experiments to explore the manifestations of **indirect cranial blast injury (icBI)**, which is believed to result from the transthoracic/transvascular mechanism of *Primary* blast injury. For these experiments, we constructed a **Thoracic-Only Blast Injury Apparatus (TOBIA)**, a modification of COBIA. TOBIA allows delivery of the blast wave directly to the thorax, with the head of rat outside of the blast chamber and away from the path of the blast wave (Fig. 7).

We studied 29 rats in 3 series: series 1 (2 rats), using peak overpressures of ~517 kPa (BDC, 24.5 cm); series 2 (21 rats), using peak overpressures of ~451 kPa (BDC, 27 cm), including 15 rats with frank exposure to TOBIA, 2 rats with the right internal jugular ligated, and 4 rats with non-impact exposure to TOBIA; series 3 (6 rats) with sham injury. The experiments in series 1 with the greater peak overpressure resulted in 100% mortality; these experiments were not pursued. The experiments in series 2 and 3 resulted in no mortality, and are the source of all of the data presented in this report. All rats in series 2 and 3 were euthanized 24 hours after injury for pathological and histological analysis.

We were exploring the hypothesis that transferring kinetic energy from the blast wave to the brain by way of major blood vessels results in injury primarily to vascular structures of the brain, with cerebral veins, the weakest of the vascular elements, being the most susceptible. These experiments impact directly on our understanding of edema formation in blast-TBI. Given the importance of brain swelling as a cause of death in humans with blast-TBI, a better understanding of the mechanism(s) by which microvessels become dysfunctional is of paramount importance with critical therapeutic implications.

Apnea and lung injury. Non-lethal blast injury induced by TOBIA (451±11 kPa) was associated with apnea that lasted up to 60 seconds and that was accompanied by a commensurate reduction in O_2 saturation (Fig. 8A,B). O_2 saturation began to recover after 1 minute, but remained depressed up to 30 minutes, compared to sham injured rats (Fig. 8C). At 24 hours after injury, the lungs showed diffuse patchy hemorrhages bilaterally that were evident on both gross examination and on H&E stained sections (Fig. 8D–F).

Brain hemorrhage. Non-lethal blast injury induced by TOBIA was not associated with subarachnoid or intracerebral brain hemorrhages in any of the 12 rats examined (Fig. 8G–I). A small thin frontal subdural hematoma was identified in 1 of the 12 rats.

Perivascular neuro-inflammation

TNFα. Immunolabeling was performed 24 hours after non-lethal blast exposure from TOBIA. Veins were identified as structures >20 μm diameter that immunolabeled for laminin, were alkaline-phosphatase-negative[16] and that had a single layer of cells in their wall. Co-immunolabeling showed prominent upregulation of TNFα in perivenular tissues throughout the brain, compared to sham control (Fig. 9A–C). We quantified the abundance of TNFα in perivenular tissues. Analyzing veins in the cortex, hippocampus and hypothalamus showed significant upregulation of TNFα each location, compared to sham controls (Fig. 9D).

ED-1. We immunolabeled for ED-1, which identifies macrophages as well as activated microglia. In many but not all cases, veins that exhibited prominent upregulation of TNFα also showed ED-1-positive cells in the vessel wall outside of the endothelium (Fig. 9E–G). In some instances, the morphology of the ED-1-positive cells was more consistent with that of activated microglia than that of invading macrophages (Fig. 9G).

Conclusion.

Working with COBIA blast injury we discovered that serotonergic neurons in raphe nucleus exhibit enhanced vulnerability to the cranial blast wave. Given the importance of the 5-HT neurons of the raphe system to numerous neuropsychological functions, our observations of pathological involvement of raphe neurons attributable to COBIA blast injury suggests a possible clue that may help explain the propensity of victims of blast injury to suffer psychiatric disturbances.

Results of the experiment with TOBIA provide evidence that the transthoracic/transvascular mechanism is indeed an important mechanism of blast-TBI, and that it leads to brain injury that is distinct from that induced by direct exposure of the cranium to blast.

Objective 1b: determine the tim e course for SUR1 and TRPM4 upregulation and downregulation post-blast-TBI.

SUR1 and TRPM4 are the regulatory and pore-forming subunits, respectively, of the SUR1-regulated NC_{Ca-ATP} channel, which is the target of glibenclamide (to be studied in Objective 1c and 1d). This channel and its subunits are not normally expressed but are transcriptionally upregulated post-injury, as we recently reviewed.[2] It is thus important to determine the time course for upregulation of the channel, because this determines the treatment window during which glibenclamide treatment needs to be started. Similarly, it is important to determine the time course of downregulation, because this determines the length of time that treatment must be continued post-injury.

We performed blast-TBI using 24.5 cm³ BDC and euthanized animals at 5 different times post-injury, to study upregulation (1/2, 1, 2, 4, 8 hr) and at 5 different times post-injury to study downregulation (1, 3, 5, 7, 10 days) of the molecular components of the NC_{Ca-ATP} channel (SUR1 and TRPM4) . Sagittal sections of the rats that underwent blast

were also labeled with cell-specific markers to determine cellular localization of the SUR1 and TRPM4 protein.

SUR1 and TRPM4. upregulation after COBIA Blast

Previously we have shown that immunohistochemically we can detect SUR1 as early as 2 hours after blast-TBI SUR1 protein in microvessels in the brain stem and cerebellum . Levels of SUR1 expression further increased at 8 and 24 hours after the blast-TBI, with predominant localization in capillaries and larger vessels. Vascular localization of newly expressed SUR1 was confirmed by co-localization with laminin, a protein expressed exclusively in the vascular wall. In addition, at 8 hours after blast-TBI, upregulation of SUR1 was detected in Purkinje neurons of the cerebellar cortex. The neuronal origin of the SUR1-labelled cells was confirmed by co-localization of SUR1 with NeuN protein, which is specific for neurons. TRPM4 protein expression after blast generally followed that of SUR1. Similarly to SUR1, TRPM4 expression after blast-TBI was primarily localized in the caudal parts of the brain with little or no expression in the cerebral cortex, thalamus or hippocampus.

During last year we continued these experiments by performing Western blot and qPCR analysis of the brain tissues impacted by the blast injury. In order to compare data on the levels of expression of SUR1 and TRPM4 RNA and protein we performed uniform harvest of the tissues from different brain regions (Fig. 10). We focused on the anatomical areas of the brain positioned in the path projected from the BDCCI output , specifically: (i) underlying cortex, (ii) thalamus and hypothalamus, (iii) cerebellum, and (iv) brain stem, as depicted on Fig 10,

So far we collected tissues in triplicates for early times after blast (1/2-24 hours and continue to collect tissues for later times (3-10 days). Our preliminary analysis indicates that we can detect significant difference in the SUR1 and TRPM4 protein as early as 4 hours after blast TBI in brain stem (Fig, 11). 24 hours after blast we detect upregulation of the both proteins in brainstem and cerebellum (Fig. 12). This result corroborates data detected immunohistochemically and confirm specificity of the signals detected with SUR1 and TRPM4 antibodies.

SUR1 upregulation after TOBIA Blast

Sur1 was strongly upregulated in vascular tissues throughout the brain (Fig. 13). Laminin-positive veins showed prominent upregulation of Sur1 in endothelial cells as well as in perivenular areas (Fig. 4A–C). Quantification of venular and perivenular Sur1 in cortex, hippocampus and hypothalamus showed significant upregulation compared to sham controls (Fig. 13A–G). Microvessels in the cortex, thalamus and hippocampus also showed upregulation of Sur1 (Fig. 13H). Larger arteries at the base of the brain (anterior, middle and posterior cerebral arteries) showed little or no upregulation of Sur1 .
We studied Sur1 expression following blast exposure with TOBIA in rats after the right internal jugular vein (IJ) had been ligated. We compared Sur1 expression in the hippocampus on the ligated side to that on the non-ligated side. As shown above, Sur1 was prominently upregulated in hippocampal veins on the non-ligated side (Fig. 14A). By contrast, Sur1 expression was minimal in hippocampal veins on the side with the

ligated IJ (Fig. 14B). Quantification of Sur1 expression confirmed significantly less upregulation on the side with the ligated IJ (Fig. 14C).

We examined Sur1 expression in rats subjected to "non-impact blast", wherein the rat was positioned next to TOBIA so that the collimated blast wave would "miss" the rat. As with sham injured rats (Fig. 13D–F) and rats with a ligated IJ (Fig. 14B), Sur1 expression was minimal in the hippocampus of rats with non-impact blast exposure (Fig. 14D).

Conclusion. Overall, our data on the time course of upregulation and downregulation of SUR1 and TRPM4 protein suggest that the SUR1-regulated NC_{Ca-ATP} channel is involved in the pathogenesis of the blast-TBI as early as 2 hours after the blast and continues to be expressed up to 1 week after blast. Thus, these data indicate that newly expressed SUR1 presents itself as a potential therapeutic target for sulfonylurea drugs such as glibenclamide for up to 1 week after blast-TBI. Data on TOBIA Blast injury suggest that vascular pathology observed in Blast TBI is mediated predominately via transthoracic route.

Objective 1c: determine the effect of glibenclamide treatment on short-term outcome from blast-TBI.

Objective 1d: determine the effect of glibenc lamide treatment on long-term neurobehavioral outcome from blast-TBI

GLP. Prior to beginning the final work on Objective 1c and Objective 1d, all SOPs were written , all equipment was calibrated and certified. All personnel involved in these experiments underwent GLP Training and were certified. Training included Pharmaceutical Training seminars on GLP. GLP training was provided by Jeiven Pharmaceutical Consulting Inc. (Scotch Plains, NJ). All short term and long term outcomes to be assessed in actual GLP study were tested in the preliminary study. We are planning to conduct this experiments and analyze collected data in the next cycle of the project.

OBJECTIVE 3: in normal human volu nteers, determine the safety of oral glibenclamide as it might be used as prophylaxis against blast-TBI.

The SUR1 blocker, glyburide, in normal human volunteers. This activity has been transferred to the University of Washington, St. Louis, due to the fact that the investigator responsible for this experiment has moved there as faculty.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

During last year of the project we concentrated our efforts on the following tasks:
1) Comparison of cellular responses in the brain regions to direct delivery of the blast wave to the brain via cranial exposure versus indirect delivery of the blast wave to the brain via thoracic exposure.
2) Detailed anatomical evaluation of the cells de novo expressing SUR1 protein Correlation between SUR1 and neuronal injury.
3) Quantitative measurements of the SUR1 and TRPM4 RNA and proteins after blast TBI
4) Evaluation of the best short term and long term outcomes to be used in GLP study of the glibenclamide efficacy in blast-TBI.
5) Detailed documentation of all blast-TBI procedures to produce Standard Operating Procedures (SOP) for GLP–valid study to determine the effect of glibenclamide treatment on outcome from blast-TBI. GLP training and certification of personnel, calibration and certification of all equipment.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research

'Direct Cranial Blast, Novel Rodent Model of Blast Traumatic Injury." Seminar at Johns Hopkins University Applied Physics Laboratory,August 11,2011

"Rodent Model of Direct Cranial Blast Injury". Kuehn R, Simard PF, Driscoll I, KeledjianK, Ivanova S, Tosun C, Williams A, Bochicchio G, Gerzanich V, Simard JM. *J Neurotrauma* 2011. PM:21639724

"Molecular, Neurobehavioral and Cognitive Evaluation of the Direct Cranial Blast Injury Model." Kaspar Keledjian, Cigdem Tosun, Volodymyr Gerzanich, J. Marc Simard. 16th Annual VAMHCS Research Day, April 23 2012 at the Baltimore VA Medical Center, Baltimore, MD.

"Molecular, Neurobehavioral and Cognitive Evaluation of the Direct Cranial Blast Injury Model." Kaspar Keledjian, Cigdem Tosun, Volodymyr Gerzanich, J. Marc Simard. National Capital Area TBI Research Symposium, May 22, 2012 at Natcher Conference Center of the National Institutes of Health, Bethesda, MD.

"Blast TBI: Involment of Direct and Indirect Injury to the Brain" Military Health System Research Symposium 13-16 August 2012 , Fort Lauderdale, Florida.

"Exposure of the thorax to a sublethal blast wave causes perivascular neuro-inflammation". Simard,JM., Keledjian, K., Tosun, C., Schwartzbauer, G., Ivanova, S., Gerzanich, V. submitted: *J. Trauma Acute Care Surgery.*

CONCLUSION: Summarize the results to i nclude the importance and/or implications of the completed research and when necessary, recommend changes on futu re work to b etter address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

Overall, we discovered that serotonergic neurons in raphe nucleus exhibit enhanced vulnerability to the cranial blast wave. These data may explain the propensity of victims of blast injury to suffer psychiatric disturbances. Results of the experiment with TOBIA provide direct evidence that the transthoracic/transvascular mechanism is indeed an important mechanism of blast-TBI. We made all necessary preparations to conduct GLP study in the upcoming cycle.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine*, etc.).
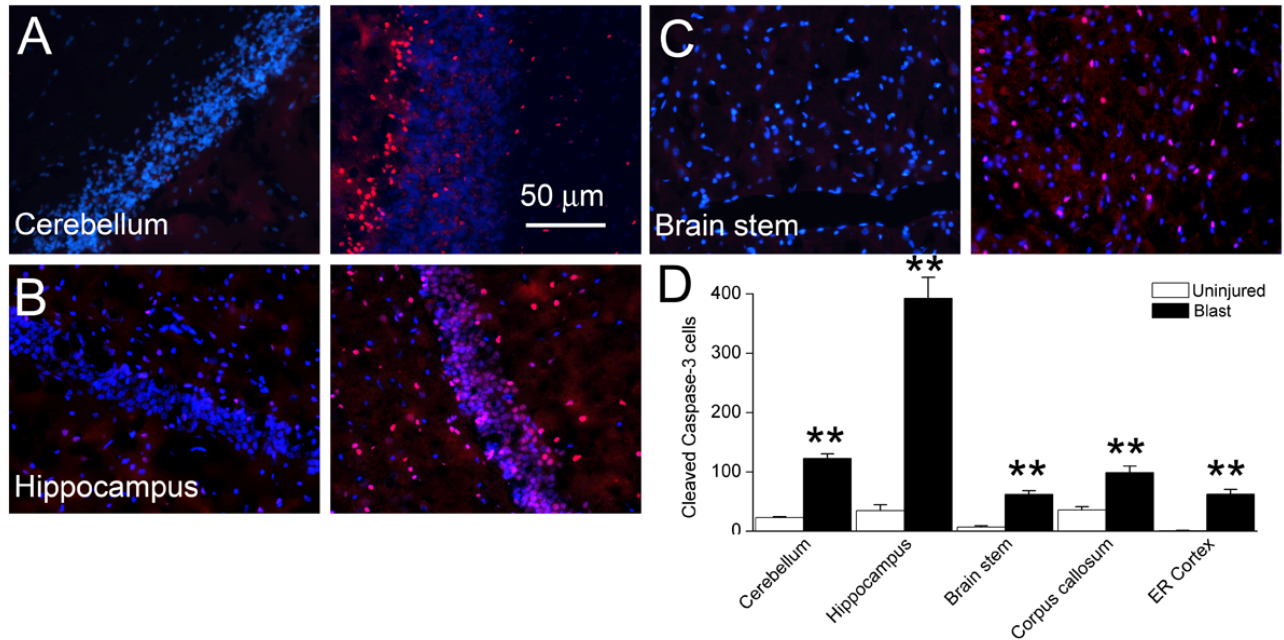
Fig. 1. Sub-lethal direct dcBI– cleaved caspase-3. A–C: Sections of cerebellum, hippocampus, and brain stem from uninjured rats (left) and from rats 24 hours after sublethal dcBI, immunolabeled for cleaved caspase-3 (red) and stained with DAPI (blue). **D:** Counts of nuclei with cleaved caspase-3 in various brain regions including the entorhinal cortex (ER), from uninjured rats (empty bars) and from rats 24 hours after sublethal dcBI (filled bars); counts were obtained in "regions of interest" (ROI) 400 x 400 µm for each area for each of 5 rats **, P<0.01.
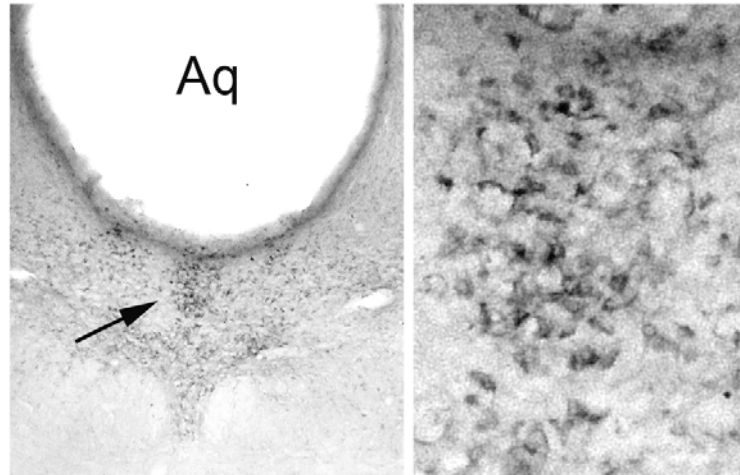
Figure 2. Brain tissues near density boundary show abnormal up-regulation of β-APP after blast. Low power view (*Left*) of the peri-aqueduct tissues shows prominent up-regulation of the β-APP in neuron-like cells. *Right:* higher power view of the area point to by arrow in left panel. Tissues labeled with anti-β-APP antibody and developed using DAB chromogen
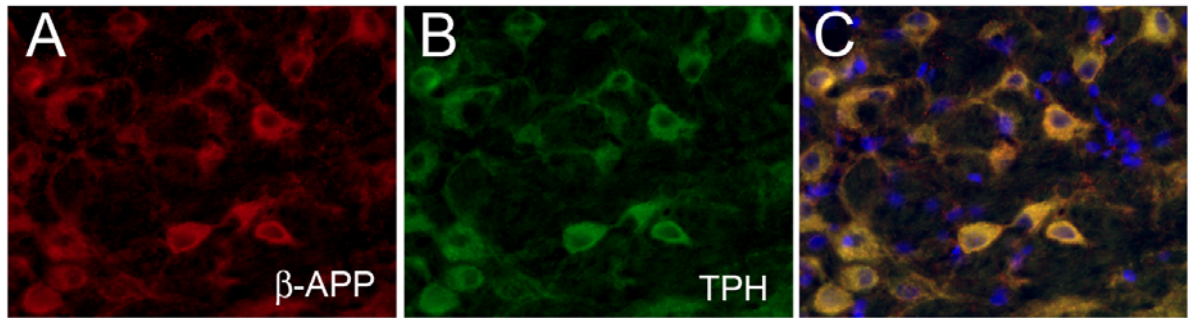
Figure 3. dcBI causes up-regulation of the β-APP in serotonergic neurons of the raphe system. Sections double labeled for β-APP (**A**, red) and Tryptohan hydoxylase (**B**, green) , enzyme involved in serotonin synthesis, and superimposed image on **C** show that raphe magnum serotonergic neurons are selectively vulnerable to the blast injury
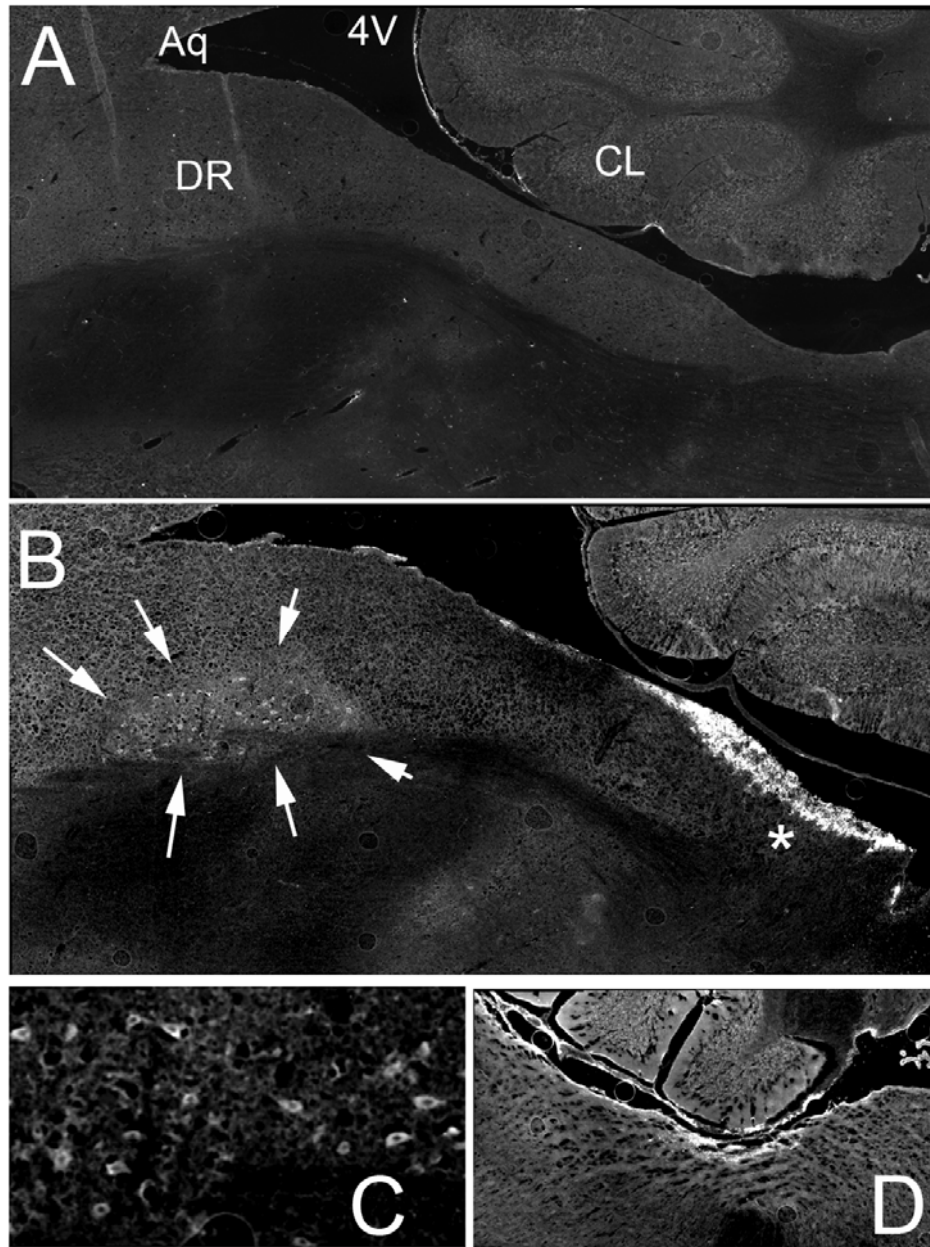
Figure 4. Brain tissues near density boundary show up-regulation of SUR1 after blast. Sagittal sections of the floor and roof of 4th ventricle from control rat brain (**A**) and brain of the rat 24 hours after dcBI (**B,C,D**) labeled for SUR1.(**Aq**-aqueduct, **4V**-4th ventricle, **CL**-cerebellar lobe, **DR**-dorsal raphe nucleus). **B**: Newly expressed SUR1 in DR is demarked by arrows and in ependyma by asterisk. **C:** High power view of the DR showing upregulation of SUR1 in individual neurons. **D :** Upregulation of SUR1 in ependyma of the 4th ventricle.
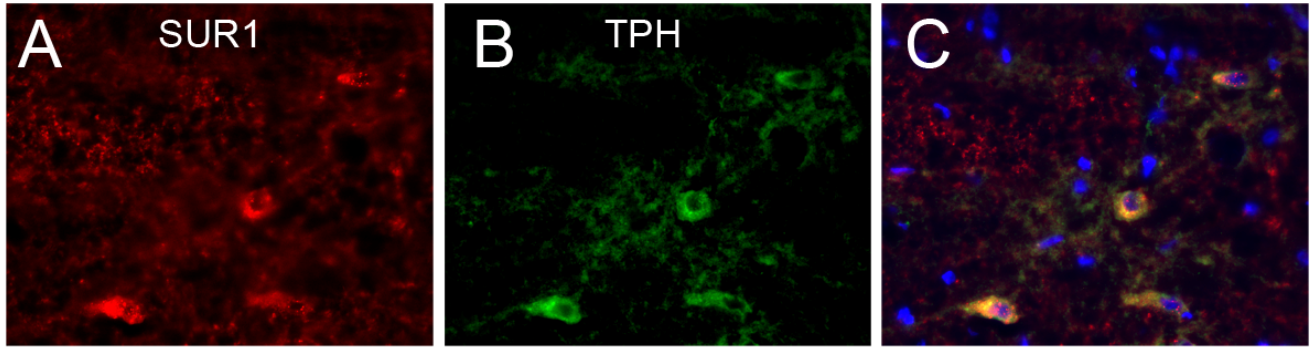
Figure 5. dcBI causes up-regulation of the SUR1 in serotonergic neurons of the raphe system. Sections from the rat brain 24 hours after dcBI double labeled for SUR1(**A**, red) and Tryptohan hydoxylase (**B**, green) , and superimposed image on **C** show that blast causes upregulation of SUR1 in raphe nucleus serotonergic neurons
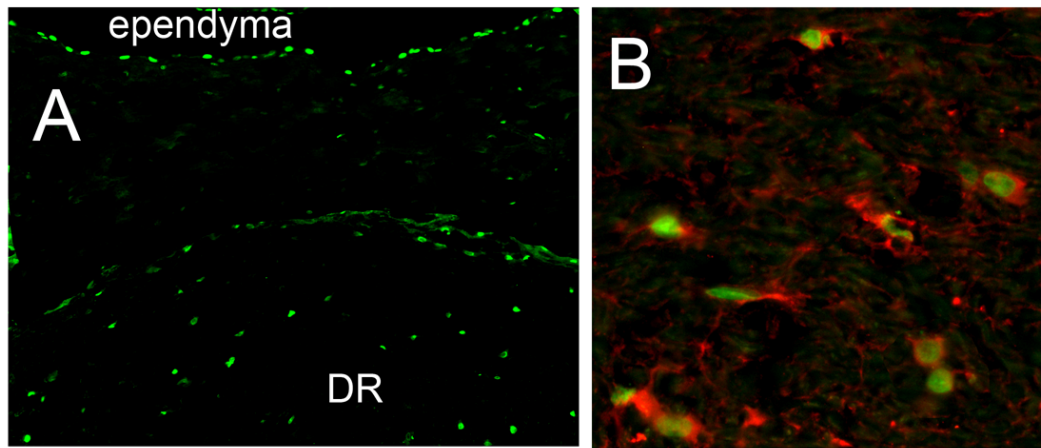
Figure 6. dcBI causes cell death in tissues near density boundary. A: Cell death 24 h after dCBI detected in ependyma of 4th ventricle and dorsal raphe nucleus (DR) using TUNEL labeling . **B**: TUNEL labeling (green) in serotonergic neurons from DR labeled for Tryptohan hydoxylase (red).
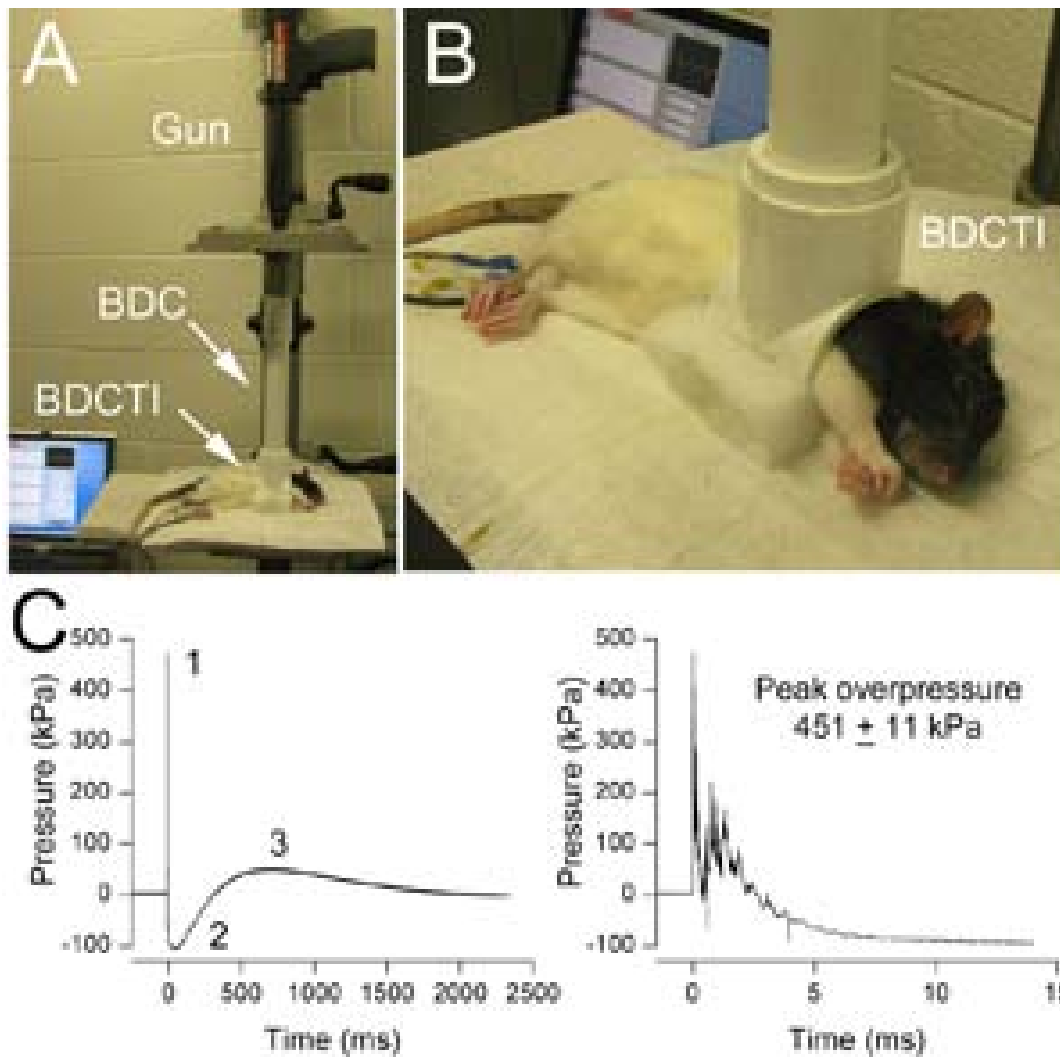
Figure 7. TOBIA and the blast wave produced by TOBIA. A,B: Overview (A) and close-up view (B) of TOBIA with the rat positioned for blast injury; BDC, blast dissipation chamber; BDCTI, blast dissipation chamber thorax interface. **C:** Blast wave produced by TOBIA shown at low and high temporal resolution; note the specific characteristics of the blast wave, including the initial brief peak overpressure (1), the underpressure (2) and the secondary slower overpressure (3), resemble closely the characteristic features of a free-field explosive blast.
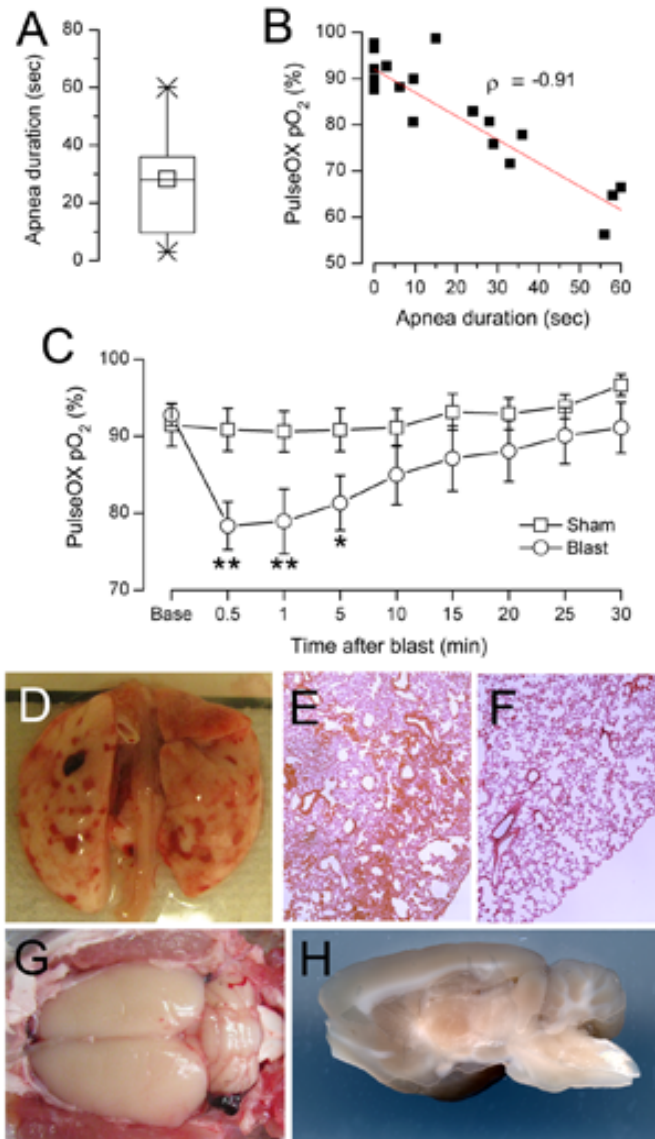
Figure 8. Blast injury induced by TOBIA is associated with pulmonary but not brain hemorrhagic injury. A,B: Box plot of duration of apnea (A) and relationship between apnea duration and O_2 saturation, measured by pulse oxymetry, in sham-injured (0 sec apnea) and TOBIA-injured rats 1 minute after blast (B); data from 5 and 13 rats in the sham and TOBIA groups, respectively; box plot symbols: box, 25th and 75th percentiles; ×, 1st and 99th percentiles; line, median; small square, mean; ρ, Pearson's correlation coefficient. **C:** Time course of O_2 saturation (mean±SE), measured by pulse oxymetry, in sham-injured (empty squares) and TOBIA-injured rats (empty circles); data from the same rats as in (A); *, $P<0.05$; **, $P<0.01$;. **D−F:** Images of whole lungs after perfusion (D) and in H&E sections after blast exposure from TOBIA (E), or after sham injury (F). **G,H:** Dorsal (G) and midsagittal (H) views of perfused brain after blast exposure from TOBIA; in (D−H), the images shown are representative of findings in the same rats as in (A).
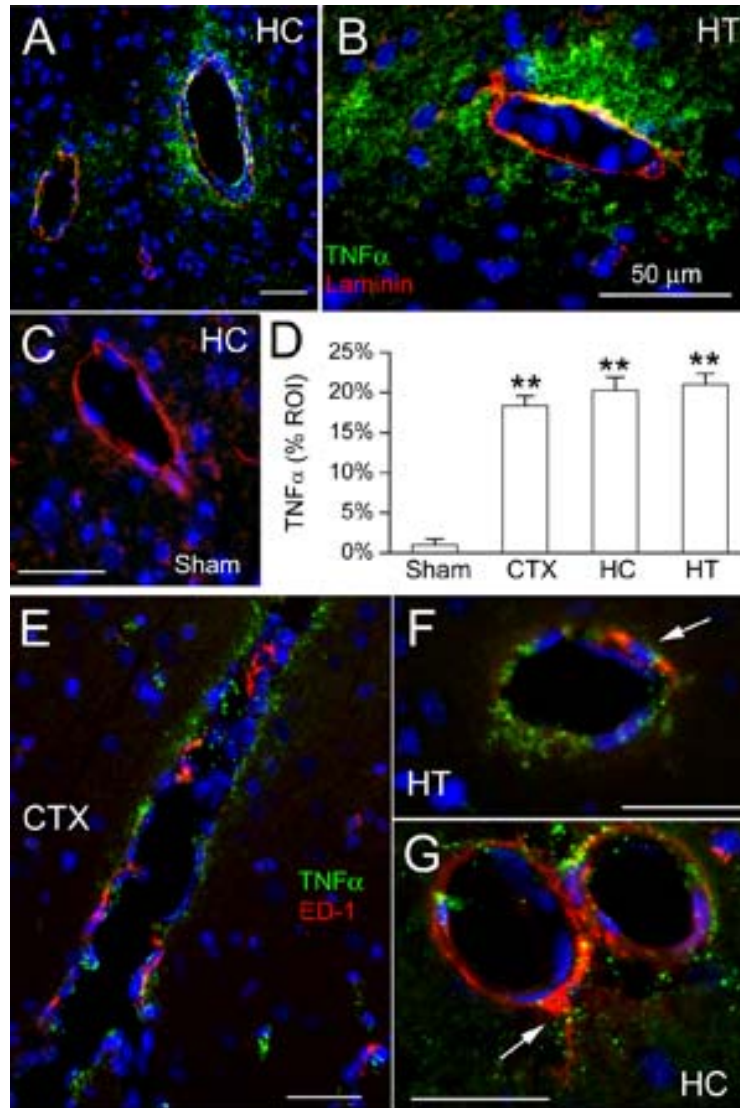
Figure 9. Blast injury induced by TOBIA is associated with perivenular neuro-inflammation. A–D: Co-immunolabeling for TNFα (green) and laminin (red) showing upregulation of TNFα in perivenular tissues after blast injury induced by TOBIA in hippocampus (HC) (A) and in hypothalamus (HT) (B), but not in a sham injured rat (C); the bar graph (D) shows the abundance of perivenular TNFα in cortex (CTX), hippocampus and hypothalamus, compared to sham (sham data from 3 regions combined); all bars, 50 μm; **, $P<0.01$; 5 veins per region from 5 rats in the sham and TOBIA groups. **E–G:** Co-immunolabeling for TNFα (green) and ED-1 (red) showing upregulation of ED-1 in the wall of vessels that also display abundant TNFα upregulation after blast injury induced by TOBIA; the arrows point to ED-1-positive cells outside of the endothelial layer, including one with a thin process typical of microglia (G); the data shown are representative of findings in the same rats as in (A–D).
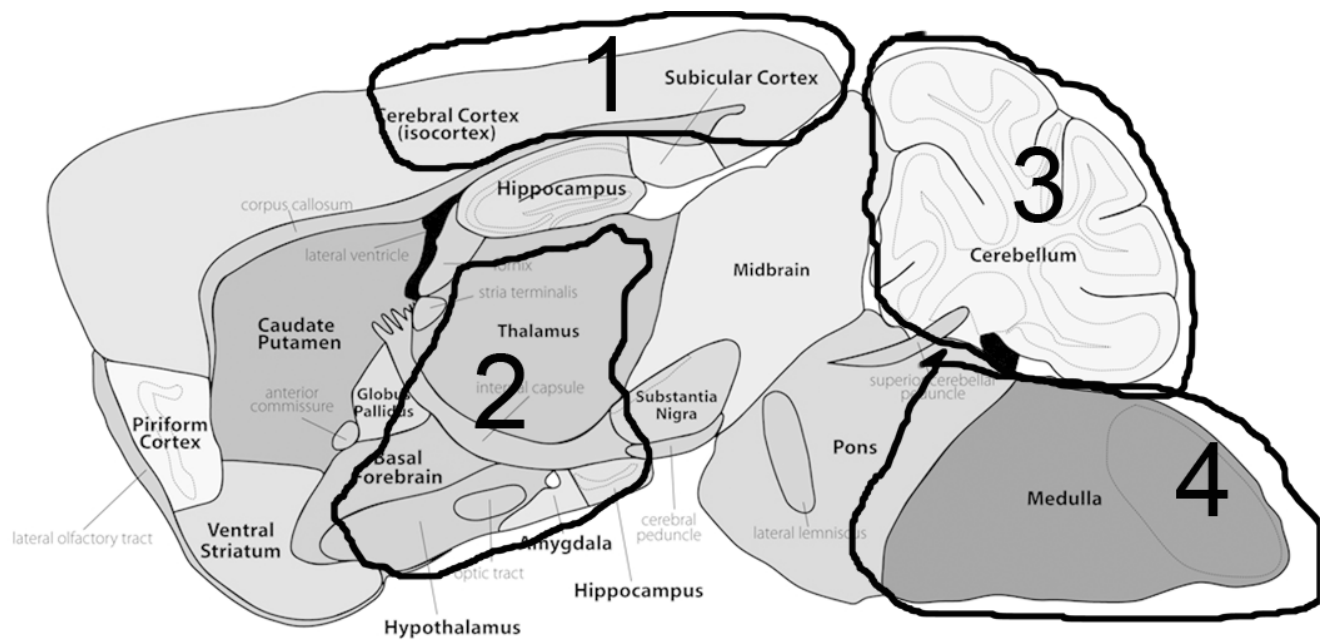
Figure 10. Rat brain anatomical regions dissected after blast injury for Western blot and PCR analysis. 1- Cortex (CTX), 2-Thalamus and Hypothalamys (Tha/HT), 3-Cerebellum (CRBL), and Brain stem (BStem).
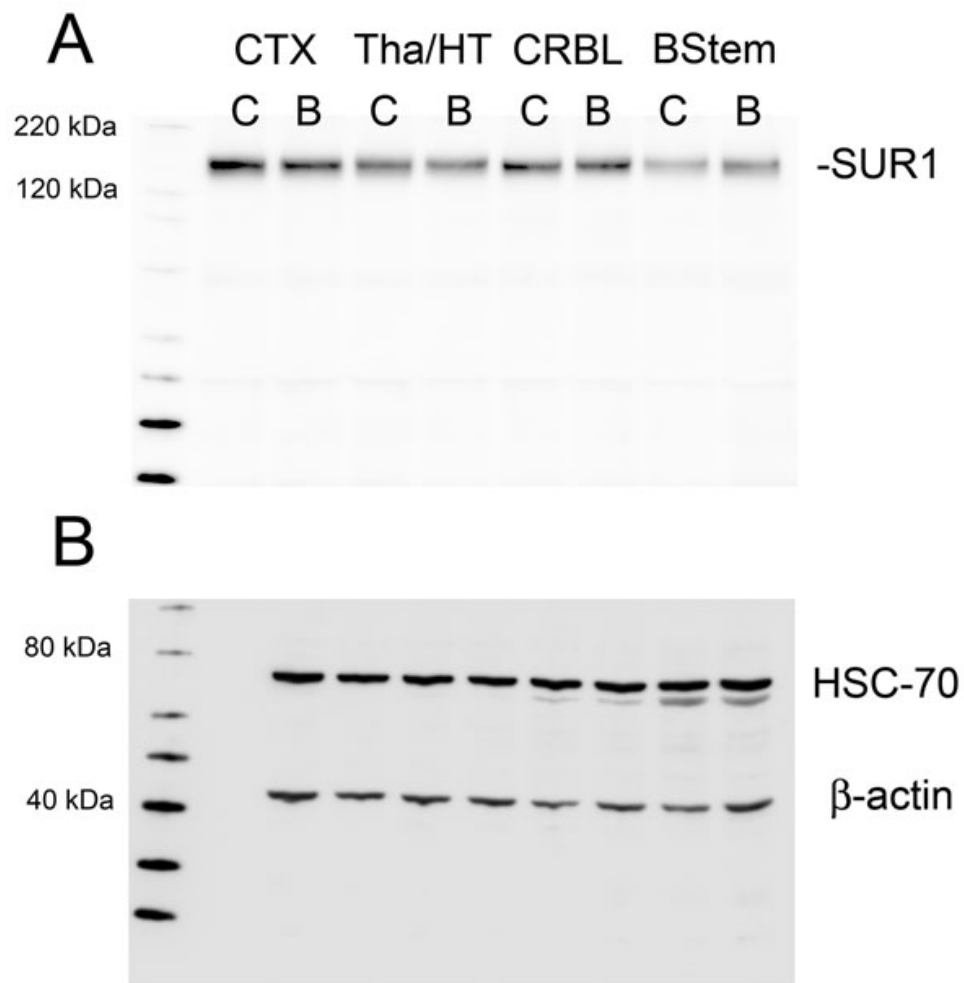
Figure 11. Western blot of the brain tissues dissected 4 hours after Blast injury. Tissues from cortex(CTX), Thalamus and Hypothalamus (Tha/HT), Cerebellum (CRBL) and Brain stem (BStem) were homogenized in RIPA buffer, run on electrophoretic gel and blotted using antibodies specific fro Sufonylurea receptor-1 (SUR1), and control proteins HSC-70 and β-actin. Note upregulation of SUR1 in brain stem.
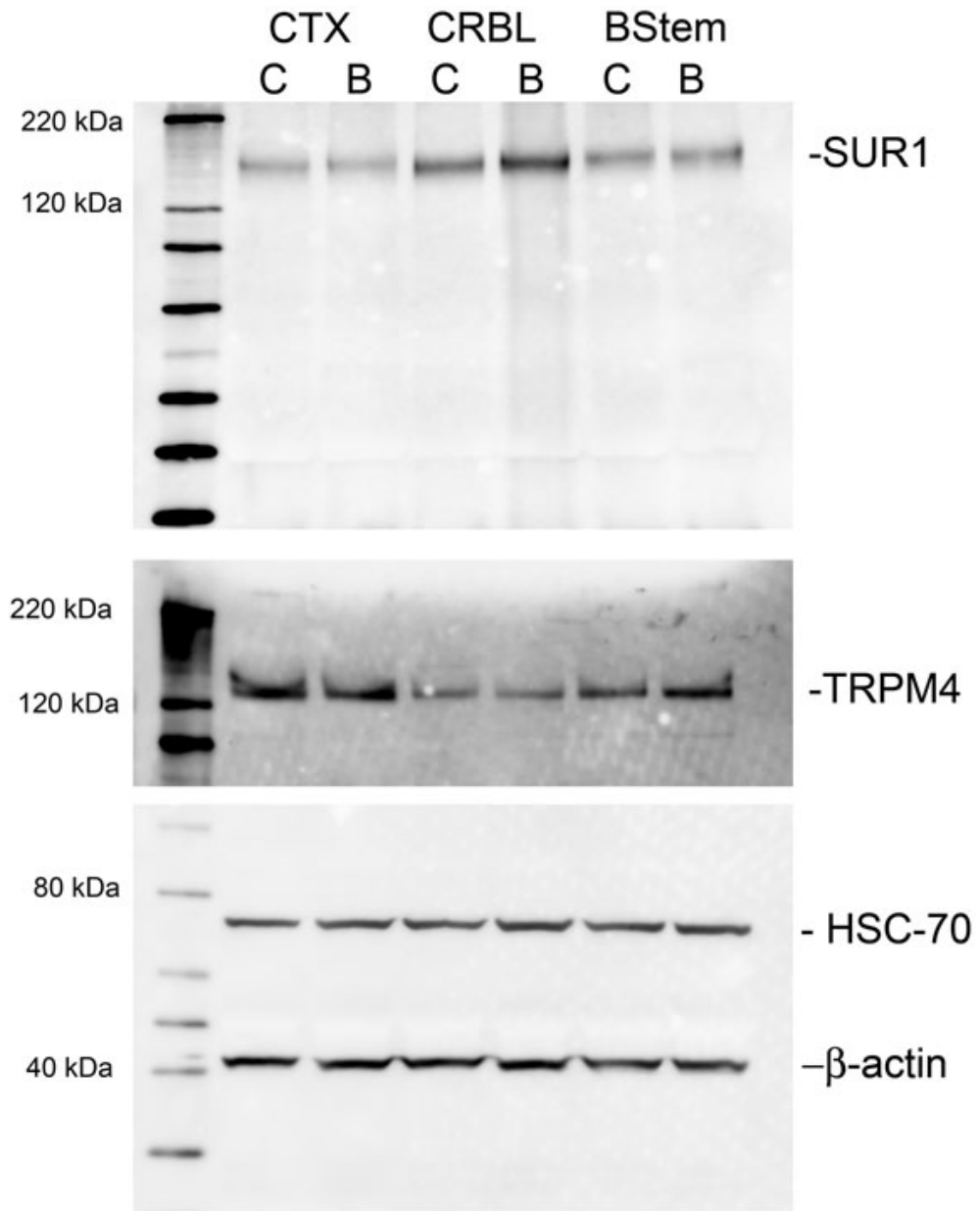
Figure 12. Western blot of the brain tissues dissected 24 hours after Blast injury. Tissues from cortex(CTX), Cerebellum (CRBL) and Brain stem (BStem) were homogenized in RIPA buffer, run on electrophoretic gel and blotted using antibodies specific fro SUR1, TRPM4 and control proteins HSC-70 and β-actin. Note upregulation of SUR1 in Cerebellum and Brain stem, and upregulation of SUR1 in Brain stem.
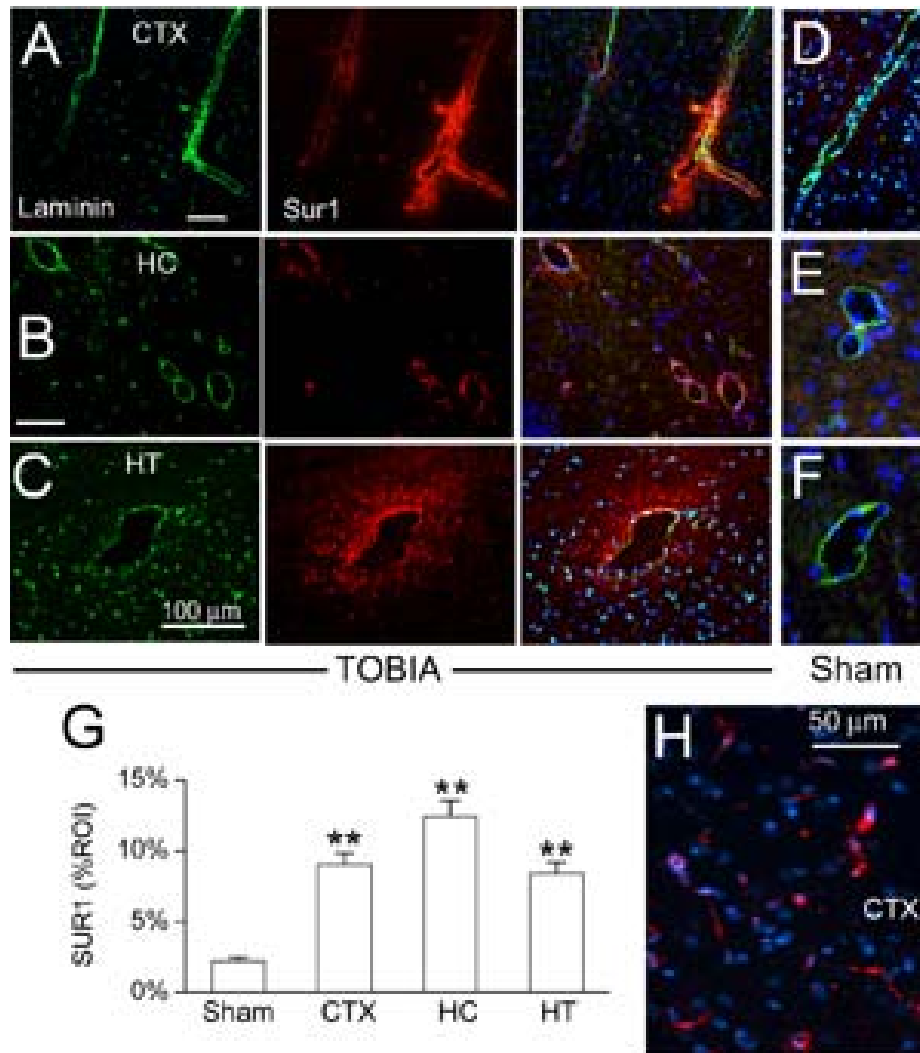
Figure 13. Blast injury induced by TOBIA is associated with perivenular upregulation of Sur1. A–C: Co-immunolabeling for laminin (green) and Sur1 (red) along with superimposed images (right), showing upregulation of Sur1 in veins and perivenular tissues in cortex (CTX) (A), hippocampus (HC) (B), and hypothalamus (HT) (C), after blast injury induced by TOBIA. **D–F:** Superimposed images of sections co-immunolabeled for laminin (green) and Sur1 (red) showing absence of Sur1 in perivenular tissues in cortex (D), hippocampus (E), and hypothalamus (F) after sham injury. **G:** Bar graph showing the abundance of Sur1 in perivenular tissues of the regions indicated after blast injury induced by TOBIA versus sham injury (sham data from 3 regions combined); 5 veins per region from 5 and 8 rats in the sham and TOBIA groups, respectively; **, *P*<0.01. **H:** Section of cortex immunolabeled for Sur1 showing Sur1 upregulation in elongated structures consistent with microvessels; the data shown in (H) are representative of findings in 3 rats.
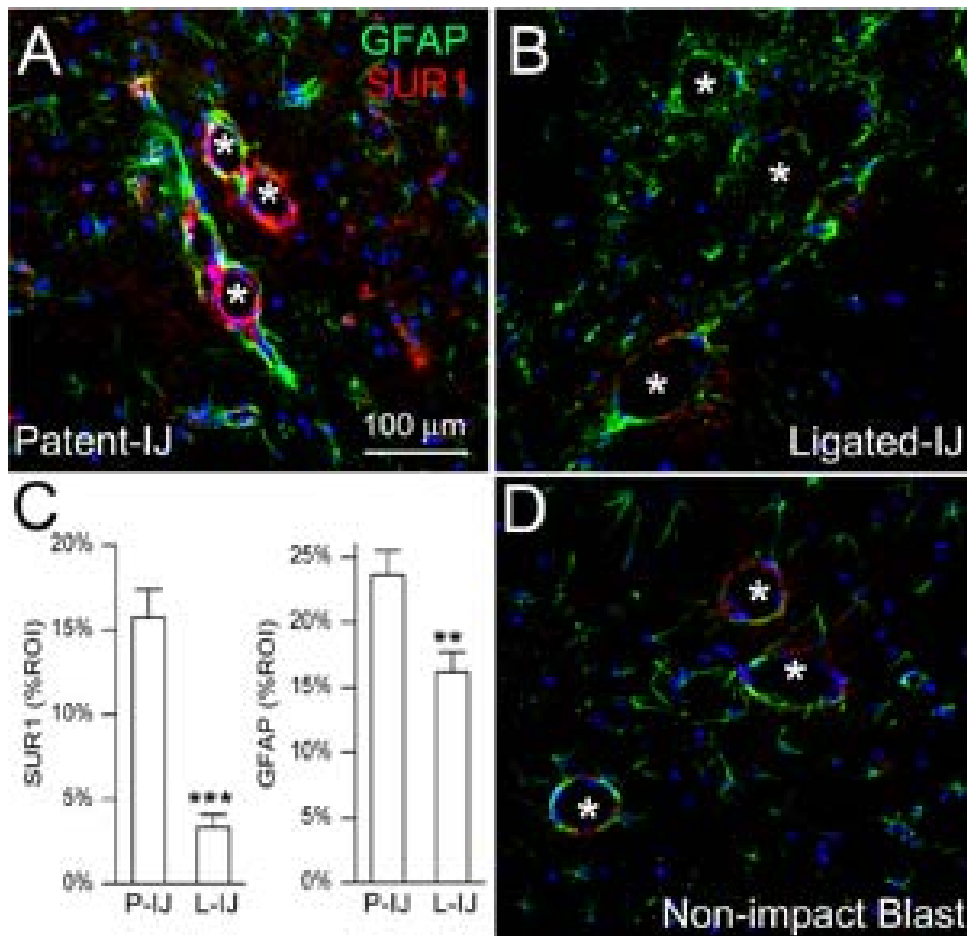
Figure 14. Perivenular injury with TOBIA requires patency of the internal jugular vein. A–C: Co-immunolabeling for GFAP (green) and Sur1 (red), showing prominent upregulation of Sur1 in veins, and upregulation of GFAP in perivenular tissues of the hippocampus on the side with a patent IJ (A), compared to weak expression on the side with a ligated IJ, after blast induced by TOBIA; asterisks denote veins; the bar graph (C) shows a quantitative analysis of Sur1 and of GFAP in or around hippocampal veins from the side of the patent (P-IJ) versus the ligated (L-IJ) internal jugular; **, $P<0.01$; ***, $P<0.001$; 17 and 26 veins from patent-IJ versus ligated-IJ sides, respectively, in 2 rats. **D:** Co-immunolabeling for GFAP (green) and Sur1 (red), showing weak expression of Sur1 in veins and of GFAP in perivenular tissues of the hippocampus following non-impact-blast by TOBIA; the image shown is representative of findings in 4 rats.
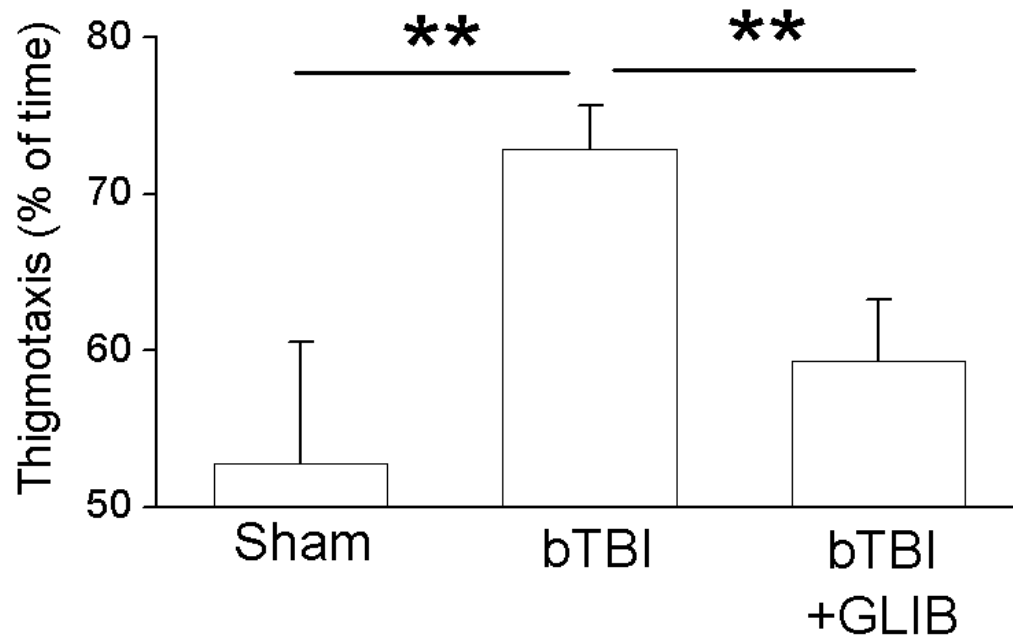
Fig. 15. Anxiety-related thigmotaxis is elevated in the rats after dcBI. Open-field thigmotaxis (tendency to remain close to the wall) times are shown for sham rats and rats 2 weeks after blast TBI with vehicle and glibenclamide treatment. Note that elevated thigmotaxis after dcBI is reduced in glibenclamide treated rats.